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PRINCIPAL INVESTIGATOR: Sabrina Ronen, Ph.D.

CONTRACTING ORGANIZATION: University of California
San Francisco, CA 94143

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Sabrina Ronen, Ph.D.

E-Mail: Sabrina.Ronen@ucsf.edu

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14. ABSTRACT

Our goal for the first year was to optimize conditions for maintaining human prostate tissue slices (TSCs) in an NMR- compatible, 3-D tissue culture bioreactor and to verify the metabolic integrity of TSCs over time. We have successfully provided a tissue culture bioreactor and optimum culture conditions and procedures to acquire good spectral quality data from metabolically viable human prostate tissue slices. In collaboration with Drs. Kurhanewicz and Peehl, we were able to acquire non-hyperpolarized and hyperpolarized $_{13}$C MR data acquired from human tissue slices that demonstrated the metabolism of the human tissue slices accurately reflects the metabolism of the *in vivo* human prostate and that [1-$_{13}$C]hyperpolarized lactate will be an accurate biomarker of prostate cancer in patients.

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Table of Contents

Page

INTRODUCTION

The overall objectives of this synergistic research is to optimize an MRS-compatible, 3D Tissue Culture Bioreactor for use with primary human prostate tissue cultures (TSCs) and use it to identify hyperpolarized molecular imaging biomarkers for improved prostate cancer patient-specific treatment planning and early assessment of response to hormone and chemotherapy. The hypotheses that will be tested in this project are that fresh human prostate tissue slices can be maintained without loss of structure, function or metabolism within a NMR compatible 3-D tissue culture bioreactor for up to three days, and that magnetic resonance spectroscopy studies of these human tissue slices can be used to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness and early response to therapy. The goals of this study will be achieved through the following specific aims. The first aim is to optimize conditions for maintaining human prostate tissue slices (TSCs) in an NMR- compatible, 3-D tissue culture bioreactor and to verify the metabolic integrity of TSCs over time. Our goal was to achieve Aim 1 in Year 1 and the focus of my role in this work was to set up the tissue culture bioreactor procedures and to evaluate varying bioreactor conditions on the structure and function of cultured human prostate tissues.

BODY

Tissue Culture Bioreactor Procedures: The preparation of the prostate tissue used for the prostate tissue cultures began immediately after the prostate had been surgically removed from the patient. First, an 8 mm diameter cylinder of tissue was harvested from a region of interest in the excised prostate. Then, the core of tissue was submerged in phosphate buffered saline (PBS) and briefly stored on ice. Next it was positioned in the center of a barrel shaped, stainless steel embedding tube that contained a warm agarose solution. The floor of the embedding tube was the sample holder/plunger for the microtome. After the entire assembly and its contents were carefully cooled on ice, the agarose solidified and adhered to the microtome plunger. The cylinder of agarose gel surrounding the tissue core attached the core to the microtome plunger as shown in Figure 1 and supported the core during the cutting process. Using a Krumdieck Tissue Slicer, a microtome manufactured specially for cutting soft tissue (Alabama Research and Development, Munford, Al), the core was sliced into 300 mm thick disks. The excess agarose was removed from the tissue slices using a cold PBS bath and the slices were either saved for subsequent histological analysis or immediately placed on stainless steel screens that were resting in a custom medium. The screens were designed to fit into the wells of a 6-well culture plate. Histology was taken from the first, middle, and last slices for each core after the slices had been preserved in optimal cutting temperature (OCT) compound, frozen, cut into 10 mm thick sections, and stained with Heomatoxylin & Eosin (H&E). Once the slicing of the tissue core was finished, 2.5 mL of fresh medium was placed in each well of a 6-well culture plate and the stainless steel screens were positioned in the wells. The 6-well plates were placed inside an incubator on a slanted surface that slowly rotated about an axis perpendicular to the surface. Since the tissue slices were raised above the bottom of the culture plate wells by the screens, the rotation of the plates cycled the slices through a period of submersion in the medium followed by exposure to air. The air was warmed to 37°C by the incubator and infused with 5% CO_2. For the preliminary 2D ^{1}H and ^{13}C HRMAS studies described in Dr. Kurhanewicz's progress report, the tissue slices used were cultured in this environment for two hours with 4 mM [3-^{13}C]pyruvate in the media.

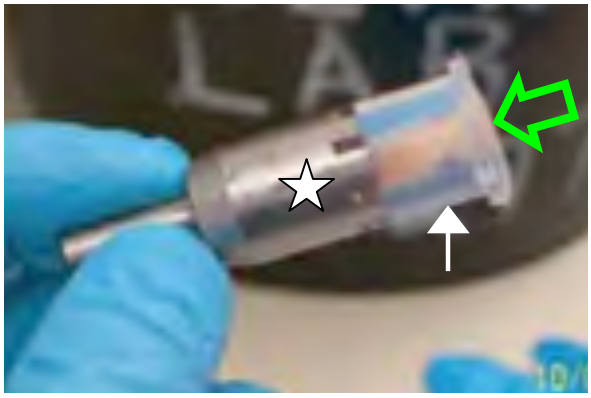

Figure 1. Photograph of prostate tissue core (Green Open Arrow) after it has been embedded in agarose gel (White Solid Arrow) to attach it to a stainless steel microtome plunger (White Star).

Preparation of Tissue Slices for 1-D and 2-D HR-MAS Studies: The tissue slices were cultured in supplemented PFMR-4a medium that was described in Dr. Peehl's progress report. However, Dr. Peehl's medium produced many unwanted peaks in the ^{1}H spectra, making it difficult to quantify the metabolites of interest. As a result, for the two hour [3-^{13}C] labeling experiments, the tissue slices were cultured in a threonine free DME medium after it was mixed

with the custom formulation of supplements as described in Dr. Peehl's progress report. In addition, the tissue culture medium was supplemented with androgens by adding 10nM R1881 to the medium. The glucose concentration was 1mM.

Once the culturing of the tissue slices was complete, they were quickly rinsed in a bath of cold PBS to remove any residual medium that was coating the tissue slices. By culturing the slices in DME and rinsing them with PBS, the ^{1}H spectra obtained from them were almost totally free of contamination (Figure 1, Kurhanewicz progress report). After the slices were carefully strained from the PBS bath, they were placed in a customized sample tube, developed specifically for the tissue slices. A sample tube (figure 2) was fabricated by modifying a transfer pipette. It was designed to form the tissue slices into a cylindrical shape with a 3mm diameter that mimicked the shape of the HR-MAS rotor. By quickly forming them into the shape of the HR-MAS rotor prior to freezing, the slices could be transferred to the HR-MAS rotor at the time of the HR-MAS experiment with minimal effort and without thawing them. Once the tissue slices were collected in the bottom of the sample tube, they were weighed and immediately frozen on dry ice. The frozen samples were stored at -80°C until they were analyzed using an HR-MAS protocol described in Dr. Kurhanewicz's progress report.

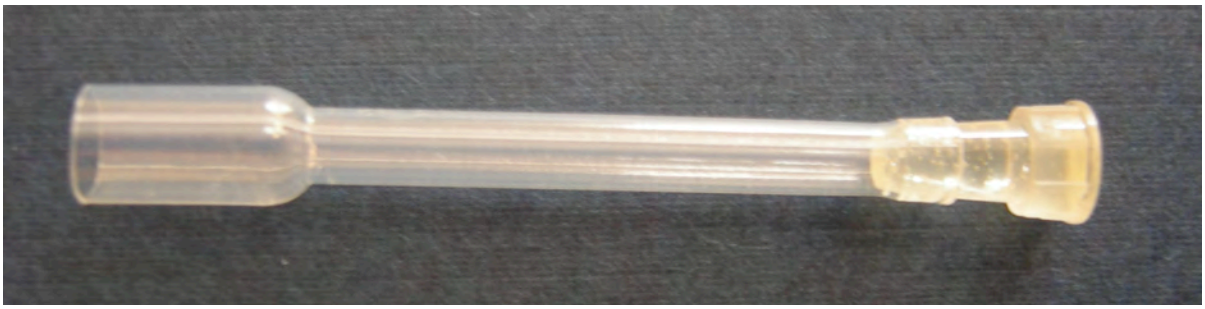

Figure 2 A photograph of a sample tube designed for storing tissue cultures. The sample tube was fabricated from a plastic transfer pipette.

Preparation of Tissue Slices for NMR Compatible Bioreactor Studies: The Prostate tissue used in these experiments was obtained from Dr. Peehl as described in her progress report. Briefly. the tissue was harvested from patients undergoing radical prostatectomy. Once the gland was removed it was given to a technician who immediately took two 8 mm cores from it. The cores were placed into chilled Hepes Buffered Saline (HBS) while the microtome was started. Adjustments were made to the microtome to ensure a thickness of 270 um, which was also rechecked using a calibration tool made specifically for the microtome. The core was then inserted into the microtome and collected. The first slice made was used for pathology while the next three slices were subsequently used for the bioreactor experiment. This was repeated ten times so that a total of ≈ thirty to forty slices were used for the experiment while slices between a set of three was used for pathology. Eleven total slices were acquired for pathology because an extra slice was taken after the tenth set of three slices was taken. This would ensure accurate pathology and tissue homogeneity for the experiment.

Once the microtome produced a set of slices for the experiment it was placed into PFMR-4A media with reduced bovine pituitary extract at a concentration of 10 ug/mL. The media also contained androgens, more specifically 10 nM of R1881. 15-mL conical tubes with the media were used to store each set of three slices with 3 mL of the complete PFMR-4A media. The slices used for pathology were placed in optimal cutting tissue solution (OCT) and frozen right away. Hematoxylin and Eosin (H&E) staining was done on the slices put on OCT and read to ensure homogenous histology for the set of tissue used in the experiment. The conical tubes were packed on ice and transported to UCSF, with an average travel time of 30 minutes. Once

the tissue had arrived to UCSF and the homogeneity of the tissue was confirmed the conical tubes encasing the tissue was left in ice while 198 mL of PFMR-4A was warmed to 37º C. Once the media was warmed the bioreactor system was set up.

A 4-channel Gilson peristaltic pump was used to power the perfusion of the system. Polyethylene tubing was used for the inflow of the bioreactor, which had an inner diameter of 0.055 inches, with a length of 8 feet from the pump. This type of tubing was used because it did not produce air bubbles when pumping from the media container. From the end of the polyethylene tubing a 23-guaged catheter was placed on which lengthen the line to 12 feet. The catheter was inserted into a 12-inch glass pipette, which was modified to protect and buttress the fragile tubing. The catheter was used to increase the velocity of media flow at the tissue so that separation of the tissue and adequate perfusion was ensured. Next the outflow lines consisted of a main outflow and an emergency outflow line. Both lines were 12 feet in length and silastic. 4 feet of polyethylene and silastic tubing was connected to the other end of the pump that was then inserted into a container with the PFMR-4a media inside. The system was autoclaved and gamma irradiated before each experiment. The NMR tube was then set with 3 mL agarose at the bottom of the tube so that the tissue would sit within the active region of the RF-coil. The tissue once placed into the 10 mm NMR tube was then connected to the outflow and inflow lines and then sealed. 2 mL of media was place inside the NMR tube while 198 mL was put into the media container giving 200 mL in total. An airline was connected to the NMR tube and inside the media. Once the system was moved to the magnet the airline was connected to a 5% oxygen and air tank. The NMR tube was placed inside the Varian 500 MHz NMR with the temperature set to 35º C. The perfusion system was then set at 4 to 6 RPM.

Serial ^{13}P and ^{13}C spectral data were acquired from 3 cancer TSCs (Gleason 3+3, 3+4 and 4+4), and 3 benign TSC's as described in Dr. Kurhanewivz's progress report. The malignant and benign ^{31}P TSC spectra obtained were identical to what has been previously published for *in vivo* ^{31}P spectra from the human prostate(1), and the ^{31}P spectra remained constant over a 32 hour time period. Additionally, pathology at the end of the bioreactor study demonstrated preservation of *in vivo* tissue structure, with pathologist's giving an average pathologic score of good (3.3 ± 0.2).

KEY RESEARCH ACCOMPLISHMENTS:

- We optimized the tissue culture media developed by Dr. Peehl in order to the maintain pathologic and metabolic viability of non-hyperpolarized [1-^{13}C]pyruvate and hyperpolarized [1-^{13}C]pyruvate prostate tissue slice (TSC) labeling studies without having interfering resonsnces in the TSC spectra.
- We developed and implemented a sample tube for storing tissue slices for HR-MAS and NMR compatible bioreactor studies during cold transport from Stanford to UCSF.
- We developed and implemented a procedure to transfer the ^{13}C labeled tissue slices into the HR-MAS rotor, while maintaining the metabolic integrity of the TSCs.
- We modified a 10 mm NMR compatible cell culture bioreactor to work for the TSC's, and developed and implemented a procedure to transfer the ^{13}C labeled tissue slices into the NMR compatible bioreactor, while maintaining the metabolic integrity of the TSCs.
- We systematically changed bioreactor condition (perfusion rate, oxygenation, media) in order to maintain the pathologic and metabolic integrity of benign and malignant human prostate tissues for 32 hours.

REPORTABLE OUTCOMES: The data reported in all three progress reports has been written up and will be submitted to the journal *Cancer Research* by August 2011.

CONCLUSION: We have successfully achieved our goals for specific aim 1: providing a tissue culture bioreactor and optimum culture conditions and procedures to acquire good spectral quality data from metabolically viable human prostate tissue slices. In collaboration with Dr. Kurhanewicz, we were able to acquire non-hyperpolarized and hyperpolarized ^{13}C MR data acquired from human tissue slices that demonstrated the metabolism of the human tissue slices accurately reflects the metabolism of the *in vivo* human prostate and that [1-^{13}C]hyperpolarized lactate will be an accurate biomarker of prostate cancer in patients. Based on successfully accomplishing the goals of aim 1, we are proceeding with aims 2 and 3.

References

1. Kurhanewicz J, Dahiya R, Macdonald JM, Jajodia P, Chang LH, James TL, & Narayan P Phosphorus metabolite characterization of human prostatic adenocarcinoma in a nude mouse model by 31P magnetic resonance spectroscopy and high pressure liquid chromatography. NMR Biomed. 1992; 5(4),185-192.

APPENDICES: N/A